



Letter to the Editor

New multiplexes for Europe—Amendments and clarification of strategic development**Abstract**

Recently, the ENFSI/EDNAP groups issued advice on the design of the next generation of STR multiplexes in order to encourage standardisation within Europe. As the result of collaborative experimentation within the EDNAP group, we demonstrated that the low molecular weight STRs had substantial benefits to detect degraded samples. We subsequently recommended adoption of three new mini-STR loci to improve the success rate of degraded DNA markers, concurrent with the reduction in size of the existing STR markers in current use. This also improves the discriminating power of the system which is important to improve the power of national DNA databases. Subsequent discussions have occurred with manufacturers and members of the ENFSI/EDNAP groups. Because significant time and investment is required to develop new multiplexes of 13+ STR loci, manufacturers indicated that it would be preferable to adopt a staged approach. Two differing, but parallel strategies have now emerged. The first strategy employs a 13 STR loci multiplex incorporating three mini-STRs into the current multiplex test. The second strategy employs a multiplex of six high molecular weight STRs (in current use), modified to provide smaller amplicons combined with an additional two loci of high discriminating power. Eventually, the two strategies will converge to provide a single multiplex of 15 STR loci. The process will be guided by the ENFSI/EDNAP groups.

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1. Introduction

A recent collaborative exercise carried out within the EDNAP group demonstrated that low molecular weight STR loci had substantial benefits to detect degraded samples [1]. Degraded samples show an increased level of DNA fragmentation due to biochemical processes occurring within the cells, therefore by targeting smaller loci there is an increased chance of successful amplification. As a consequence of this study we recommended adoption of three new mini-STR loci to improve the success rate of degraded DNA markers, concurrent with the reduction in size of the existing STR markers in current use [2]. As a result of further discussions with the ENFSI group meeting held at Kenilworth, Warwickshire, UK, on 26–27 October and within the EDNAP group, and in consultation with representatives of multiplex STR manufacturers we have decided on the following amendments to our paper [2].

2. Evaluation

- (1) From the recent publication of Coble and Butler [3] it is clear that locus D14S1434 has a low discriminating power (DP) compared to existing STRs currently in use (approximately one in seven for the white Caucasian population). Locus D2S441 has a much better DP, hence we recommend substitution of D14S1434 with D2S441. This triplex will have a combined DP of 1 in ca. 2500. This will also mean that new multiplexes will have two loci situated on the same chromosome (D2S1338 and D2S441), however they are on different arms and separated by many mega-bases, hence independent Mendelian inheritance is assured. Otherwise the list of loci remains exactly the same. We now believe that there is sufficient information available to preclude the possibility of any further future alterations regarding the addition of new loci. It is our stated aim to encourage and to work closely with manufacturers to build these new multiplexes for the market.

Table 1

List of additional loci in two groups where group I are mini-STRs (70–125 bp) and group II comprise D12S391 and D1S16565 midi-STRs (120–180 bp) and TPOX (which could be designed as a mini-STR)

Group I	Group II
D10S1248	D12S391
D22S1045	D1S1656
D2S441	TPOX

Note that D2S441 now replaces D14S1434. TPOX will be subject of further discussion and is currently held in reserve only.

The new loci to be included to the core loci are in two groups (Table 1).

- (2) After extensive discussions with manufacturers it is clear that there are significant challenges to produce multiplexes in accordance with our original requirements. Whereas the objective is essentially unchanged, it is clear that the process of development will be facilitated if a staged approach is adopted. This has benefits, both from the point of view of the manufacturer, and the EDNAP/ENFSI groups since it will allow careful evaluation at each stage of development and will allow feedback to assist the development of the next iteration.
- (3) It is clear that there are differing requirements within Europe that necessitate two different but parallel strategies. These differing requirements arise mainly from differences in both the emphasis of the type of evidential material that is routinely analysed and in laboratory practices. For example, there are special analytical requirements to analyse low level highly degraded DNA from volume crime (e.g. burglary), automation, and the need for a single test strategy in some laboratories. However, there is full agreement that the overriding requirement is that both strategies must converge to achieve the aim of increasing the number of universal (Interpol) loci to be used throughout Europe. Otherwise it will not be possible to efficiently compare samples when the European database eventually comprises millions of samples.

3. Proposed multiplex strategies

The two strategies are as follows (Fig. 1).

3.1. Strategy 1

Some laboratories require a new multiplex that combines their existing multiplex in current use along with the mini-STRs in group I. The simple addition of three new loci into the low molecular weight region of existing multiplexes means that the technical challenges are reduced and can be accomplished much more easily. This will reduce timescales

for implementation. Laboratories that prefer this strategy will typically wish to work with a single multiplex for all evidential types and will routinely analyse a significant proportion of highly degraded samples. The new multiplex comprises the group I loci along with the multiplex loci in current use. This enables immediate inclusion of three new loci into the national DNA databases of countries using this system. This is particularly important for countries that have several million samples in their databases in order to reduce the chance of adventitious matches. The intention is that the new multiplex will quickly replace the current multiplex. It is not intended as an adjunct.

Casework success rates (because of the inclusion of mini-STRs <125 bases) and discriminating power will both increase.

3.2. Strategy 2

Other laboratories place greater priority upon development of a multiplex where existing high molecular weight loci are reduced in size. It does not matter if this is a system that does not comprise all of the loci that are in current use in national DNA databases in the first iteration. It is envisaged that it will be used in conjunction with existing multiplex systems rather than acting as a replacement. In addition many laboratories have expressed a wish to include group II loci already validated by the EDNAP group (D1S1656 and D12S391) [4] in the preliminary multiplex design. These are midi-STRs (120–180 bp). The advantage of these loci is a high discriminating power (ca. 1 in 150). There is strong view that these two loci should be brought forward or prioritised in the first iteration of the new system. The other locus in group II is TPOX. At present the use of this locus will be the subject of further discussion and possible amendment, since this locus has relatively low discriminating power.

The new multiplex under strategy 2 is not a replacement for the existing multiplex; it is an adjunct. An advantage of the approach is that it will allow a good opportunity for concordance studies, since two multiplex systems will usually be run in parallel.

4. Convergence of the two strategies

The convergence of strategies 1 and 2 will evolve via a series of steps, to produce a final product containing 15 loci. The EDNAP/ENFSI groups will work closely with the manufacturing companies to advise on each iteration of a new multiplex. In addition there will be local requirements to consider, such as the inclusion of locus SE33 for German laboratories. However, local requirements are beyond the scope of these recommendations.

The precise format of the new multiplexes is for the manufacturing companies to decide in relation to marketing surveys, and feasibility studies. The success of this (unfunded) project will rely upon close co-operation of all

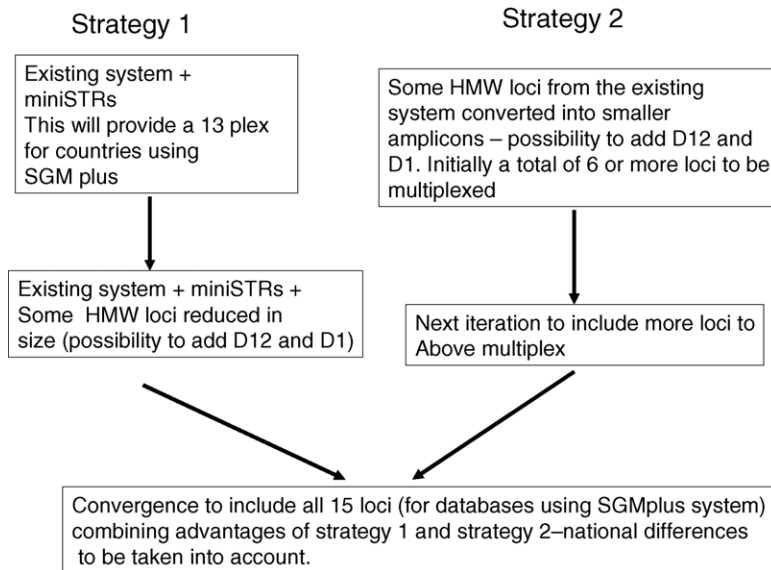


Fig. 1. Two multiplex strategies, showing path to convergence.

concerned. Our intention is to design multiplexes that will significantly benefit criminal justice systems by simultaneously increasing detection rates and reducing potential adventitious matches. This will be achieved by decreasing the amplicon sizes and increasing the number of loci detected, thereby increasing the chance of success in highly degraded samples and increasing the resulting discrimination power of all samples tested.

References

- [1] L.A. Dixon, A.E. Dobbins, H.K. Pulker, J.M. Butler, P.M. Vallone, M.D. Coble, W. Parson, B. Berger, P. Grubwieser, H.S. Mogensen, N. Morling, K. Nielsen, J.J. Sanchez, E. Petkovski, A. Carracedo, P. Sanchez-Diz, E. Ramos-Luis, M. Brion, J.A. Irwin, R.S. Just, O. Loreille, T.J. Parsons, D. Syndercombe-Court, H. Schmitter, B. Stradmann-Bellinghausen, K. Bender, P. Gill, Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise, *Forensic Sci. Int.*, doi:10.1016/j.forsciint.2005.11.011.
- [2] P. Gill, L. Fereday, N. Morling, P.M. Schneider, The evolution of DNA databases—recommendations for new European STR loci, *Forensic Sci Int.* (2005) (e-pub. online July).
- [3] M.D. Coble, J.M. Butler, Characterization of new miniSTR loci to aid analysis of degraded DNA, *J. Forensic Sci.* 50 (2005) 43–53.
- [4] P. Gill, E. d'Aloja, B. Dupuy, B. Eriksen, M. Jangblad, V. Johnsson, A.D. Kloosterman, A. Kratzer, M.V. Lareu, B.

Mevag, N. Morling, C. Phillips, H. Pfitzinger, S. Rand, M. Sabatier, R. Scheithauer, H. Schmitter, P. Schneider, I. Skitsa, M.C. Vide, Report of the European DNA Profiling Group (EDNAP)—an investigation of the hypervariable STR loci ACTBP2, APOA11 and D11S554 and the compound loci D12S391 and D1S1656, *Forensic Sci Int.* 98 (1998) 193–200.

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